## MODIFICATION OF THE ALL PROTOCOL TO CHARACTERISE THE OVERALL ANT ASSEMBLAGE IN TEMPERATE EUCALYPT FOREST

#### DAVID BRAY

## 260 Gilloglys Rd, Bulga Forest, NSW 2429 (Email: davidmbray@yahoo.com.au)

#### Abstract

A standardised sampling procedure for ants, the ALL (Ants of the Leaf Litter) Protocol, was modified for use in temperate eucalypt forest. Terrestrial samples from leaf litter and pitfalls (the basic ALL Protocol) were supplemented with arboreal samples from tree traps. Four 200 m transects were sampled using the modified protocol within a 0.5 ha site on a forested property located near the mid-north coast of New South Wales. Site vegetation was mixed-age eucalypt forest, which had been undisturbed for more than 30 years. Tree traps captured a total of 54 species and 19 of these were absent from terrestrial samples. The addition of tree traps to the basic ALL Protocol: i) increased the number of species detected per species occurrence by an average of 20% per transect, with almost 2/3 of the increase due to species of Camponotus, Polyrhachis and Myrmecia; ii) increased the proportion of common species collected in transects, with less variation in species numbers between transects; and iii) provided a more comprehensive characterisation of this ant assemblage. Three groups of ants were present in the assemblage: 1) widespread species, active both in litter and on trees; 2) small litter-dwelling terrestrial species; and 3) larger species which were captured primarily on trees but were mostly ground-nesting. The genera of ants detected at the site were largely shared with similar east coast sites. Voucher specimens were deposited at the Australian Museum and images of the ants are available by email from the author.

## Introduction

Australian ants have been extensively used as bioindicators to monitor environmental changes (Majer et al. 2004) and also as a target group in biodiversity surveys (Stanisic et al. 2005, Burwell and Nakamura 2011, Callan et al. 2011). However, thorough assessments of ant biodiversity are time-consuming and often involve specialist entomologists (Andersen et al. 2002). In an effort to make invertebrate assessments easier to conduct, rapid survey procedures have been developed using simplified methods for sampling, sorting and identification of specimens (Oliver and Beattie 1996). Andersen et al. (2002) simplified the assessment procedure by sorting only larger ant species from 12 selected genera and thereby reduced the survey effort while achieving similar conclusions to those of a more intensive survey. A rapid sampling procedure, the Ants of the Leaf Litter (ALL) Protocol, has been proposed for assessing ant assemblages of the forest floor from samples collected by pitfall trapping and extraction of ants from leaf litter using mini-Winkler sacks. This standardised sampling methodology enables direct comparisons between sites and between studies (Agosti and Alonso 2000). The ALL Protocol was developed for tropical forests, where distinct assemblages of arboreal and litter ants occur, but there is little evidence for this distinction in temperate forests (Gotelli et al. 2011). In Australian eucalypt forests few ant species are known to nest and forage primarily in trees, but ground-nesting species may forage in trees (Andersen

and Yen 1992) and some of these species appear to do so preferentially when leaf litter is well developed on the ground (Andersen 1995). Ants which forage largely in trees and strictly arboreal species are both likely to be under-represented in terrestrial samples collected with the basic ALL Protocol. Several ant surveys with a focus on the overall ant assemblage in tropical habitats have included tree traps to sample the arboreal stratum (Andersen *et al.* 2006, Andersen *et al.* 2007).

The present study examined the use of baited tree traps to supplement ground samples collected with the basic ALL Protocol in a temperate eucalypt forest and thereby provide a less biased description of the overall ant assemblage. A relatively undisturbed site with a well developed leaf litter layer was surveyed using multiple transects and the modified ALL Protocol. Results from individual transects were inspected for changes in species richness and species composition due to the addition of tree-trap samples.

## Methods

## Study site

The study site was located on a 400 ha property on the eastern fall of the Great Dividing Range, near the NSW mid-north coast (31.513°S, 152.246°E, 500 m altitude, mean annual rainfall 1150 mm). The property was almost entirely forested and surrounded by similarly forested lands. The topography consisted of ridges with moderate slopes. Disturbances over the past century included cattle grazing, timber harvesting and frequent fires. Prior sampling had indicated the presence of a relatively rich ant fauna on a sheltered slope with north-west aspect which had been free from these disturbances for more than 30 years and this was selected for the study area. The study site was located mid-slope and limited to 0.5 ha to avoid changes in soil moisture and ground vegetation on the upper slope and lower slope.

Site vegetation consisted of dry eucalypt forest, with an open mixed-age canopy dominated by Grey Gum (*Eucalyptus propinqua*), Grey Box (*E. moluccana*) and Forest Red Gum (*E. tereticornis*). The open understorey was mostly Black Oak (*Allocasuarina littoralis*) and the shrub layer was sparse. The closed ground cover consisted of grasses (*Themeda, Entolasia*), Matrush (*Lomandra*), sedges (*Gahnia*) and a well-developed leaf litter layer of 2-5 cm depth. The soil was finely textured, compact and stony, with patches of surface stones, gravel, logs, branches and debris.

## Sampling

Four linear 200 m transects (A-D) were sampled with the modified ALL Protocol at the study site during January and February, 2011. Successive transects were displaced at least 10 m to avoid overlap. Sampling stations were located at 10 m intervals along each transect. Terrestrial samples from litter and a pitfall, together with an arboreal sample from a tree trap, were collected at each station (a total of 20 samples per method per transect).

Each leaf litter sample was collected from an area of  $1 \text{ m}^2$ , sieved to remove coarse material and placed in 5 mm mesh plastic containers which were suspended in mini-Winkler sacks for 48 hours (Bestelmeyer *et al.* 2000) at temperatures of 17-38°C. Emergent ants were collected in 80% methylated spirits. Pitfalls and tree traps were constructed from plastic cups, 65 mm diameter and 90 mm depth, partly filled with 80% methylated spirits as collecting fluid and operated for 48 hours. Pitfalls were dug into the ground and shaded with plastic containers. Tree traps were pinned to a tree trunk at 1.5 m height and baited around the rim with honey (Fig. 1). Observations of ant nests were recorded as: 1) in the ground or in wood on the ground; or 2) above ground in dead wood of standing trees.



Fig. 1. Tree trap attached to the trunk of a canopy eucalypt by upholstery pins, partially filled with 80% methylated spirits and baited around the rim with honey.

Ants were identified to genus using keys by Shattuck (1999) and CSIRO (2012), then separated into morphospecies (referred to as species hereafter) and identified to described species or species-group where suitable keys were available. Voucher specimens were deposited at the Australian Museum and images of all morphospecies detected are available by email from the author.

## Data analysis

Performance of the modified ALL Protocol was qualitatively assessed from the increase in the number of species collected and the change in species composition in each transect relative to data from the terrestrial samples of the basic ALL Protocol.

At each sampling station within transects, data from litter and pitfall samples were pooled for the basic ALL Protocol and this was pooled with the data from the tree traps for the modified ALL Protocol (*i.e.* 20 pooled-method samples per transect). Incidence data were used as it has been advocated as a more appropriate unit of ant biodiversity than the abundance of individual ants (Ellison *et al.* 2007, Gotelli *et al.* 2011). Incidence (occurrence) was recorded as the presence or absence of a species in each sample of pooled-methods data or in each sample collected by individual methods.

The numbers of species and species occurrences were recorded and the software EstimateS 8.2 (Colwell 2009) was used to compute species accumulation curves for transect data. Species detected were plotted against species occurrences rather than samples, to reduce bias due to differences in ant numbers (Gotelli *et al.* 2011). The number of species collected in each transect was compared at a value of species occurrences corresponding to the least total for any transect. At this value, the number of species in each transect was estimated, where necessary, by interpolation between points in the species accumulation data.

Changes in species composition were identified by inspection of the incidence data collected by the basic and modified ALL Protocols for each transect.

## Results

## Species richness

The numbers of species occurrences, genera and species collected were greatest in litter samples and least in pitfalls (Table 1). Transects varied in the numbers of species occurrences and species detected, with greater variation between transects collected with the basic ALL Protocol than with the modified ALL Protocol (Fig. 2).

Data from tree traps supplemented basic ALL Protocol data by an average of 84 species occurrences, three genera and 14 species per transect (Table 1). The number of species collected in each transect ranged from 47 to 60 for the basic ALL Protocol and 61 to 70 for the modified ALL Protocol (Fig. 2), representing differences between transects of up to 28% and 15% respectively.

The modified ALL Protocol accumulated more species per species occurrence than the basic ALL Protocol. Transect B with basic ALL Protocol yielded 309 species occurrences, the least number for any transect and the

data for the remaining transects were standardised to this value. At 309 species occurrences, transects with the basic ALL Protocol averaged 51 species and this increased by 20% to 61 species with the addition of data from the tree traps used in the modified ALL Protocol (Table 1).

Table 1. Species occurrences at the site. Totals for each sampling method are the combined data for four transects. Average values per transect (based on the four transects) compare the basic (Bas.) and modified (Mod.) ALL Protocol using: a) raw data; and b) data standardised to 309 species occurrences.

	Species occurrences	Genera	Species
Totals			
Litter	1216	39	79
Pitfalls	265	24	39
Tree traps	485	° 25	54
Average/transect			
a) raw data			
Bas. ALL Protocol	331	31	52
Mod. ALL Protocol	415	34	66
b) standardised to 309 sp. occ.			
Bas. ALL Protocol	309		51
Mod. ALL Protocol	309		61

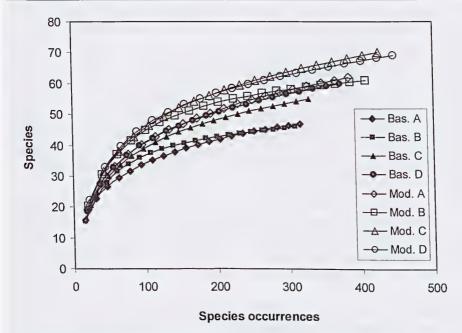


Fig. 2. Species accumulation curves (based on species occurrences) for each of the four transects (A-D) using data from the basic ALL Protocol (Bas.) and modified ALL Protocol (Mod.).

# Composition of the ant assemblage

Ant species detected at the site are listed in Table 2. The species composition of the assemblage in terrestrial samples (litter and pitfalls) differed from that in arboreal samples (tree traps). A total of 54 species was collected in tree traps (Table 1). Terrestrial samples collected 46 species not captured by tree traps; another 35 species were present in both strata and 19 species were captured only in tree traps (Table 2). Ten subfamilies of ants were present in terrestrial samples, while tree traps contained ants from only five subfamilies (Table 2). Differences in species composition between ants collected with the basic and modified ALL Protocols were most conspicuous in the three subfamilies Formicinae, Myrmicinae and Myrmeciinae. The modification to the ALL Protocol added totals of 11, four and three species, respectively, to these subfamilies (Table 2). Twelve of the 19 species unique to tree traps were from the genera Camponotus, Polyrhachis and Myrmecia (Table 2). The modification to the ALL Protocol added an average of 8.5 species per transect from these three genera, representing 61% of the overall increase in species numbers.

The subfamilies Myrmicinae and Formicinae accounted for 68% of species collected from transects and 71% of species occurrences. The Myrmicinae were comprised largely of small species active in leaf litter, with few of these species active on tree trunks. The Formicinae consisted of a mixture of small species which were primarily active in the ground litter and large mobile species which were active on tree trunks but rarely captured on the ground.

Genera which were common in samples from one stratum, but not the other, included *Pheidole*, *Stigmacros*, *Hypoponera*, *Solenopsis* and *Lordomyrma* in terrestrial samples and *Camponotus*, *Polyrhachis*, *Myrmecia* and *Leptomyrmex* on tree trunks. Genera that were common both on the ground and on tree trunks were *Anonychomyrma*, *Nylanderia*, *Meranoplus* and *Crematogaster*. The genus *Rhytidoponera* included two similar species with differing habitat preferences: *Rhytidoponera victoriae* (André) was common only on the ground, while *Rhytidoponera metallica* (Smith) was common both on the ground and on tree trunks.

Forty-four of the collected species were considered common as they occurred at more than 10% of sampling stations (*i.e.* incidence greater than 8 in the Mod column in Table 2). Of these 44 common species, at least 42 (95%) were collected along each of the four transects with the modified ALL Protocol, while each transect with the basic ALL Protocol collected at least 36 (82%).

Ant nests were observed on or near the site for 23 of the 54 species active on tree trunks and nests of 18 of these species were in the ground or in wood lying on the ground, while those of five species were arboreal in dead standing trees of *Eucalyptus*, *Acacia* and *Allocasuarina* (Table 2).

**Table 2.** Total number of species occurrences (incidence) in 80 samples each from: L, litter; P, pitfalls; T, tree traps; **Bas**, basic ALL Protocol; and **Mod**, modified ALL Protocol. Nest sites (N) for species active on trees are denoted as: G, in the ground or in wood on the ground; or A, above ground in standing dead wood.

Species	L	Р	Т	Bas	Mod	N
Amblyoponinae						
Amblyopone sp. 1	3	0	0	3	3	
Prionopelta robynmae Shattuck	1	0	0	1	1	
Stigmatomma sp. 1	1	0	0	1	1	
Cerapachyinae						
Cerapachys turneri Forel	5	0	0	5	5	
Cerapachys larvatus (Wheeler)	4	0	0	4	4	
Sphinctomyrmex sp. 1	1	0	0	1	1	
Dolichoderinae					-	
Anonychomyrma sp. 1	50	33	33	61	72	G
Anonychomyrma sp. 2	6	2	1	6	6	0
Iridomyrmex splendens Forel	7	5	18	10	21	G
Iridomyrmex mayri Forel	3	4	6	6	12	G
Leptomyrmex nigriventris (Guérin)	0	2	18	2	19	A
Ochetellus sp.1 (glaber group)	1	0	0	1	1	11
Ochetellus sp.2 (glaber group)	1	0	3	ĩ	4	G
Tapinoma sp.1	35	0	2	35	36	0
Tapinoma sp.2	0	0	1	0	1	
Ectatomminae						
Rhytidoponera metallica (Smith)	14	13	25	25	39	
Rhytidoponera victoriae (André)	56	-36	3	63	63	G
Formicinae			-	05	05	0
Acropyga myops Forel	1	0	0	1	1	
Camponotus aeneopilosus Mayr	3	2	24	4	28	G
Camponotus elegans Forel	1	0	46	.1	46	G
Camponotus sp.6 (intrepidus group)	0	0	26	0	26	G
Camponotus sp.7 (? humilior)	0	0	21	Ő	20	G
Camponotus sp. 9 (near elegans)	0	0	3	0	3	0
Camponotus sp.12 (sponsorum group)	0	0	1	Ő	1	
Camponotus macrocephalus Erichson	0	0	1	Õ	1	
Melophorus sp. 1	1	0	0	1	1	
Melophorus sp. 2	2	· 0	1	2	3	G
Notoncus capitatus Forel	25	15	17	33	37	0
Nylanderia sp. 1	0	0	5	0	5	Ă
Nylanderia sp. 3	58	3	59	59	71	Λ
Paraparatrechina sp. 2 (minutula group)	38	3	4	38	40	G
Paraparatrechina sp. 4 (minutula group)	26	1	2	27	27	U

Species	L	Р	Т	Bas	Mod	N
Polyrhachis sp. 2	0	0	1	0	1	G
Polyrhachis phryne Forel	2	0	26	2	27	
Polyrhachis sidnica Mayr	0	0	5	0	5	
Polyrhachis sp. 11	1	0	3	1	4	Α
Polyrhachis sp. 15	0	0	1	0	1	
Polyrhachis sp. 16	0	0	3	0	3	
Prolasius sp. 1	14	1	5	15	19	
Prolasius sp. 2	51	5	2	54	54	
Prolasius sp. 3	44	6	7	44	46	
Prolasius sp. 4	2	1	0	3	3	
Prolasius sp. 5	2	0	0	2	2	
Pseudonotoncus hirsutus Clark	0	0	1	0	1	
Stigmacros sp. 1	19	0	0	19	19	
Stigmacros sp. 2	12	0	7	12	17	G
Stigmacros sp. 4	24	0	0	24	24	
Stigmacros sp. 5	38	1	0	38	38	
Stigmacros sp. 6	1	0	1	1	2	
Stigmacros sp. 8	18	1	0	18	18	
Heteroponerinae						
Heteroponera sp. 1 (imbellis group)	29	5	0	33	33	
Myrmeciinae						0
Myrmecia nigrocincta Smith	1	1	12	2	14	G
Myrmecia brevinoda Forel	0	0	3	0	3	G
Myrmecia sp.3 (gulosa group)	0	0	1	0	1	
Myrmecia fulvipes Roger	1	0	0	1	1	
Myrmecia sp.9 (mandibularis group)	0	0	1	0	1	
Myrmicinae	22	1	0	23	23	
Carebara sp. 1	1	1	0	2	2	
Colobostruma alinodis Forel	1	0	0	1	1	
Colobostruma lacuna Shattuck	20	2	33	21	43	Α
Crematogaster sp. 1		12	2	21	24	
Crematogaster sp. 2	14	0	3	3	6	
Crematogaster sp. 6	3		1	0	1	
Epopostruma wardi Shattuck	0	0		47	47	
Lordomyrma sp.1	47	0	0	8	8	
Mayriella spinosior Wheeler	5	3	0	2	6	
Mayriella sp. 2 (near abstinens)	2	0	4	1	1	
Mayriella abstinens Forel	1	0	0	49	54	G
Meranoplus sp. 1	43	26	17		10	0
Mesostruma browni Taylor	10	0	0	10	1	G
Monomorium rubriceps Mayr	0	0	1	0	1	

Species	L	Р	Т	Bas	Mod	Ν
Monomorium tambourinensis Forel	40	1	0	40 -	40	
Monomorium sydneyense Forel	3	0	4	3	7	
Monomorium fieldi Forel	7	5	4	11	14	Α
Monomorium sp. 7 (? sydneyense)	1	1	0	2	2	
Monomorium leae Forel	1	0	0	1	1	
Orectognathus phyllobates Brown	1	0	0	1	1	
Orectognathus antennatus Smith	0	0	1	0	1	G
Orectognathus rostratus Lowery	7	0	0	7	7	
Orectognathus sp. 5 (? clarki)	2	0	5	2	7	
Pheidole sp. 1	55	35	4	65	66	
Pheidole sp. 2	63	14	0	66	66	
Pheidole sp. 4	1	0	0	1	1	
Pheidole sp. 6	2	0	• 0	2	2	
Pheidole sp. 7	0	1	2	1	3	
Podomyrma sp. 2	0	0	1	0	1	
Solenopsis sp. 1	53	12	0	55	55	
Solenopsis sp. 2	2	2	0.	4	4	
Solenopsis sp. 3	22	1	0	23	23	
Strumigenys perplexa Smith	33	0	0	33	33	
Strumigenys sp. 2	11	0	0	11	11	
Tetramorium confusum Bolton	6	1	4	7	11	
Ponerinae						
Hypoponera sp. 1	76	5	0	77	77	
<i>Hypoponera</i> sp. 2	3	0	0	3	3	
<i>Hypoponera</i> sp. 3	1	0	0	1	1	
Leptogenys sp. 1	2	1	0	3	3	
Pachycondyla sp. 1	12	1	0	13	13	
Pachycondyla sp. 2	1	0	0	-1	1	
Ponera leae Forel	5	0	0	5	5	
Proceratiinae						
Discothyrea sp. 1	34	0	0	34	34	
Discothyrea sp. 2	1	0	0	1	1	

# Discussion

# Evaluation of the modified ALL Protocol

In this study the ant assemblage in a temperate eucalypt forest was sampled with the terrestrial sampling methods of the basic ALL Protocol supplemented by arboreal samples collected with baited pitfall traps on tree trunks. The modification to the ALL Protocol yielded a modest increase in the number of species detected, a clearly identifiable change to the species composition of the ants collected in each transect, and less variable estimates of species richness and species composition.

The addition of tree traps increased the number of species collected in each transect and some of this increase can be attributed to the extra species occurrences generated by the tree traps. However, when the data were standardised to species occurrences the modified ALL Protocol collected an average of 20% more species per transect, indicating that it accumulated species more efficiently than the standard ALL Protocol despite the increase in sampling effort. The additional field time required for the modified ALL Protocol was minimal as the tree traps were installed and operated concurrently with the ground pitfalls.

The composition of the ant assemblage collected with the standard ALL Protocol was partly altered by the addition of tree traps. Substantial changes were confined to the subfamilies Formicinae and Myrmeciinae, in which the number of species collected increased by 50% and 250% respectively. The additional species were mostly from Camponotus, Polyrhachis and Myrmecia, genera which were infrequently present in the unbaited pitfall and litter samples collected with the standard ALL Protocol. These results appear support the observations of Andersen (1995) that subordinate to Camponotini, such as Camponotus and Polyrhachis, avoid well developed litter by foraging in trees. However, these ants may have been present and active on the ground, but not readily collected by unbaited pitfalls in the well developed litter cover at the site. Ants are more likely to be captured in pitfalls when the surrounding ground cover has a relatively open structure (Melbourne 1999). Dense litter cover reduces pitfall capture rates (Bestelmeyer et al. 2000) and Andrew et al. (2000) found that Camponotus was more common on the ground at burnt rather than unburnt forest sites. The use of bait may also influence the capture rate for pitfalls as Romero and Jaffe (1989) captured more ant species in savanna habitats when pitfalls were baited with meat, although Wang et al. (2001) reported that unbaited pitfalls were more effective in temperate oak forest. Greenslade and Greenslade (1971) collected more Camponotus ants when pitfalls were baited with syrup. Thus, the probability of detecting any Camponotus species that is active on the ground may improve if honey bait is added to the ground pitfalls used in the basic ALL Protocol. Other baits, such as meat or fish, have potential to collect additional species as Kaspari and Yanoviak (2001) found that canopy ants in a tropical forest preferred meat baits to sugar baits.

Although individual transects collected with the modified ALL Protocol did not detect all species collected at the site they captured a greater proportion of the common species than transects with the basic ALL Protocol. The number of species per transect differed less between transects collected with the modified ALL Protocol than between those collected with the basic ALL Protocol. Together, these results indicated that the modified ALL Protocol provided less variable estimates of species richness and species composition than the basic ALL Protocol.

For studies which aim to maximise the species inventory of an overall ant assemblage, the improved sampling efficiency of the modified ALL Protocol should outweigh the slight increase in field time required to implement the tree traps.

# The ant assemblage

Features of the ant assemblage at the study site were the large number of species active on tree trunks and the proportion of these which were underrepresented in terrestrial samples. Previous studies indicated limited and patchy arboreal ant activity in southern Australian eucalypt forests (Majer 1990), although 37 species were recorded as active in eucalypt canopies in New South Wales (Majer *et al.* 2000) and 44 species were detected in the canopy of mallee eucalypts in northwestern Victoria (Andersen and Yen 1992). In the present study, 54 species were present in tree trap samples and a third of these were absent from the ground samples collected with the basic ALL Protocol.

The overall assemblage was comprised of three groups of species: 1) those widespread and common in both strata; 2) those primarily in terrestrial samples; and 3) those primarily in arboreal samples. Species in the widespread group included ants in a range of sizes from the subfamilies Dolichoderinae, Formicinae, Ectatomminae and Myrmicinae and they were from functional groups described by Andersen (1995) as comprising species which are usually abundant and unspecialised. The terrestrial group was the most diverse and consisted mainly of small cryptic species of Formicinae, Myrmicinae and Ponerinae, which are typical of habitats with well developed leaf litter (Andersen 1986, 1995, Hoffmann and Andersen 2003). The arboreal group was characterised by larger species of Formicinae and Myrmeciinae, which have been reported to be predominantly ground nesting (Andersen and Yen 1992). Within this group only five of the 23 nests observed during the present study were located above ground. Although ground nests of Camponotus were found up to 10 m distant from canopy trees, these ants were uncommon or rare in ground samples but more commonly collected on tree trunks. The arboreal group represented a substantial proportion of the species present and the combination of terrestrial and arboreal samples provided a more comprehensive inventory of the ant assemblage at this eucalypt forest site.

The genera found at the site were similar to those found elsewhere in eastern Australian eucalypt forests, with the majority also occurring 40 km to the east at Bulls Ground State Forest (York 2000, Andrew *et al.* 2000) and at forest

sites near Brisbane (Stanisic *et al.* 2005). However, shared genera can mask differences in species composition and to facilitate comparisons of species composition, Callan *et al.* (2011) retained reference specimens and provided online images of all ant species detected in their study at Barrow Island.

Ecological studies typically employ small plots and generate data which may not be immediately applicable at larger scales (Andersen 1997). However, the use of standardised sampling, as in the modified ALL Protocol, enables baseline data to accumulate from successive studies and provides the potential for comparative analysis at both local and regional scales. The results of this study support the use of multiple sampling methods for biodiversity assessment in order to offset the bias of individual methods and to improve detection rates for species which utilise more than one habitat.

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